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Growth and reproductive performance of locally isolated brackishwater rotifer (*Brachionus plicatilis*) feeding on different microalgae

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Abstract
Population growth and reproductive capacity of brackishwater rotifer, *Brachionus plicatilis*, were evaluated, for a period of 8 days in a temperature controlled (≈25°C) microalgal laboratory, under three different algal feeding regimens. The algal species that were tested are: (i) *Chlorella* sp. (T_1), *Tetraselmis chui* (T_2), *Nannochloropsis oculata* (T_3). The feeding density of each algal species was maintained similar as of $4.5x10^6$ cells ml. The rotifer fed on *T. chui* showed the highest ($p<0.05$) population growth (131.5 ind./ml), compared to that fed on *Chlorella* sp (45.67 ind./ml) and *N. oculata* (43.44 ind./ml). The abundance of egg bearing rotifers was also higher (35.77%) with *T. chui* than with *Chlorella* sp (27.76%) and *N. oculata* (24.60%). The results of the present study indicate that *T. chui* could be the most suitable algal food for the stock culture of locally isolated rotifer *B. plicatilis*.

Key words : Microalgae, Rotifer, Growth, Reproductive performance

Introduction

The rotifer, *Brachionus plicatilis* is an important and essential food item in the early larval stages of many marine and brackishwater fish species. It is an excellent first food for larvae because of its (i) relatively smaller size, (ii) slow swimming speed, (iii) habit of staying suspended in the water column, and (iv) ability propagation in captivity at high density and reproductive rate. Microalgae comprise the principal food component for most cultured rotifer. Many species of algae may be used and the choice may depend on the availability, ease of culture under local conditions and the exact nutritional requirements of the rotifers and target aquaculture species. Microalgae high in □-3 HUFAs such as *Nannochloropsis* sp. have been regarded as very good food for culturing rotifers (Hirata 1989, Fulks and Main 1991). The most commonly used algal species that are being used to culture rotifer are *N. oculata, Tetraselmis tatrathele, T. suecica* and *Isochrysis galbana* (Person-Le Ruyet 1975), *Chlorella* sp. (Theilacker and McMaster 1971) Highly concentrated *Chlorella vulgaris* and freeze-dried algae have also been used successfully to feed rotifers in Japan (Hirayama *et al.* 1989). *Tetraselmis subcordiformis*, *Chlorella* sp., *Chlamydomonas* sp. and *Nitzschia closterium* have been found excellent

foods for growth and hatching of *B. plicatilis*, although the green algae *Tetraselmis* was better than the others in mass culture (Wang and Liang 1980).

Though the seed production of marine shrimp, *Penaeus monodon*, has already reached to a level of self sufficiency in Bangladesh, that of many important marine and brackishwater finfishes has yet to support the commercial production. Besides the lack of technical know-how of breeding and larval rearing, knowledge on culture and supply of appropriate live food for the first feeding fish larvae is scanty. In a series of studies on live food culture for marine and brackishwater fin and shellfish, the present experiment was conducted to determine the feeding effect of three different locally available microalgal species on the growth and reproductive performance of locally isolated brackishwater rotifer, *Brachionus plicatilis*, under stock culture condition.

Materials and methods

The stock culture of the rotifer was done in a temperature controlled (about 25°C) microalgal laboratory at the Brackishwater Station of Bangladesh Fisheries Research Institute (BFRI), Paikgacha, Khulna. Three different microalgae, *viz.*, *Chlorella* sp. (T_1), *Tetraselmis chui* (T_2), and *Nannochloropsis oculata* (T_3) were used as food to evaluate their effects on growth and reproductive performance. Nine 500 ml Erlenmeyer flasks were divided into three treatment groups. The flasks were placed at a distance of approximately 30 cm from a fluorescent tube light, having an intensity of about 2500 lux/m^2/sec. Photoperiod was maintained as 16:8 hrs L:D. *Chlorella* sp., *Tetraselmis chui*, and *Nannochloropsis oculata* were collected, isolated and cultured separately in laboratory conditions using the BFRI microalgae culture medium (Shah *et al.* 2004). When the concentration of each algae reached to 4.5×10^6 cells/ml, three rotifer culture flasks were filled in up to 300 ml with each algal culture following completely randomized design.

Rotifers were collected and isolated from the local brackishwater rivers and shrimp ponds using a zooplankton net of 250 to 90μ mesh. Each of the test flasks was inoculated with rotifer at a density of 5 ind./ml. The salinity of microalgae and rotifer culture water was 20‰. The initial abundance of initial egg bearing rotifers was estimated to about 12.53%. The duration of algal feeding trial on growth and reproductive capacity on rotifer was 8 days.

The concentration of rotifers was estimated every two days. Depending upon rotifer density, an unspecified amount of rotifer culture was poured through a 45μ sieve to catch a large number of rotifers on the screen. These were then rinsed with a small amount of filtered seawater using a squirt (wash) bottle and placed in to petri dish. These concentrated rotifers were then sampled with a bulbed pasteur pipette and placed on the Sedgewick-Rafter counting cell and counted under the dissecting microscope (Braley 1994). The mean count was obtained from 6 counts. Instantaneous growth rate (K) was calculated from the expression of -

$$K = \frac{\text{Log}_e N_t - \text{Log}_e N_0}{t}$$ (James *et al.* 1983)

Where, K = instantaneous growth rate;
N_0 = initial number of rotifers; and
N_t = final number of rotifers after t days.
t = culture days

While the growth of rotifer was estimated under microscope, the number of egg bearing rotifers was also recorded. The percent abundance of egg bearing rotifers (EBR) was estimated, to understand the reproductive capacity (RC) of rotifers, following the formula given below:

$$\text{Reproductive capacity (RC)} = \frac{\text{Number of egg bearing rotifers}}{\text{Total number of rotifers}} \times 100 \text{ (Braley 1994)}$$

Data were analyzed following one way ANOVA and Duncan's Multiple Range Test (DMRT), using a PC equipped with STATAGRAPHICS *Ver. 7.*

Results and discussion

Population growth of rotifer, *Brachionus plicatilis*, fed on different microalgal diets is given in Table 1. Among the three microalgae, *Tetraselmis chui* (T_2) resulted in the significantly highest mean population growth of 131.5 ind./ml ($p < 0.05$) at the end of the eight days culture. The population growth of rotifers fed on *Chlorella* sp (T_1) and *Nannochloropsis oculata* (T_3) was not only similar ($p > 0.05$) with the growth values of 45.67 ind./ml and 43.44 ind./ml, respectively, but also nearly three times lower than that fed on *T. chui*. In 2 - 6 days of culture, the rotifers fed on *Chlorella* sp. and *N. oculata* showed apparently higher percent abundance of carrying eggs compared to those fed on *T. chui* (Table 1). However, at the 8th day of culture, the egg bearing rotifers (EBR) was significantly higher ($p < 0.05$) in those fed on *T. chui* (35.77%) than in those fed on *Chlorella* sp. (27.76%) and *N. oculata* (24.60%).

Table 1. Growth and reproductive capacity of rotifer, *Bracchionus plicatilis*, fed on different microalgal diets for an eight-day culture

Microalgal diets (Treatments)	Time in days	Population growth		Reproductive capacity	
		Initial no. (ind./ml)	Final no. (ind./ml)[1]	Initial EBR (%)	Final EBR (%)[1]
Chlorella sp (T_1)	2	5	17.26 ± 5.05	12.53	16.18 ± 4.15
	4		28.33 ± 4.50		18.26 ± 5.50
	6		34.20 ± 6.23		21.85 ± 5.75
	8		45.67 ± 7.65[b]		27.76 ± 5.85[b]
Tetraselmis chui (T2)	2	5	29.82 ± 5.84	12.53	13.58 ± 6.46
	4		58.91 ± 4.78		14.56 ± 6.78
	6		88.76 ± 5.45		15.39 ± 4.45

	8		131.5 ± 7.32[a]		35.77 ± 5.30[a]
Nannochloropsis oculata (T3)	2	5	16.63 ± 7.25	12.53	15.19 ± 5.85
	4		26.63 ± 5.50		19.89 ± 6.55
	6		29.82 ± 6.60		20.50 ± 5.75
	8		43.44 ± 5.15[b]		24.60 ± 6.14[b]

[1]Dissimilar superscripts in column denote differences at 5% level of significance ($P<0.05$).

The results clearly indicate that, among the three microalgae, *T. chui* possesses the dietary superiority over *Chlorella* sp. and *N. oculata* in culturing *B. plicalities*. The highest population growth and reproductive capacity in rotifers feeding on *T. chui* might be due to its comparatively larger cell size and better nutritional quality than the other two microalagal species (Hoff and Snell 1989). The incremental growth rate in rotifer population feeding on *T. chui* was progressively higher and steady (Fig. 1), indicating significant dietary role in propagation of rotifer in controlled conditions. Rotifers fed with *T. chui* had higher instantaneous growth rates compared to rotifers fed on *Chlorella* and *N. oculata* (Fig. 2). The trend in instantaneous growth rates in rotifers indicates that *T. chui* also might have beneficial effects in early growth phase in brackishwater rotifer population. However, the instantaneous growth rates of rotifers fed on different microalgae under the present culture conditions was higher than that of 0.133, which has been observed in rotifers fed on Chlorella sp. and baker's yeast (James *et al.* 1983).

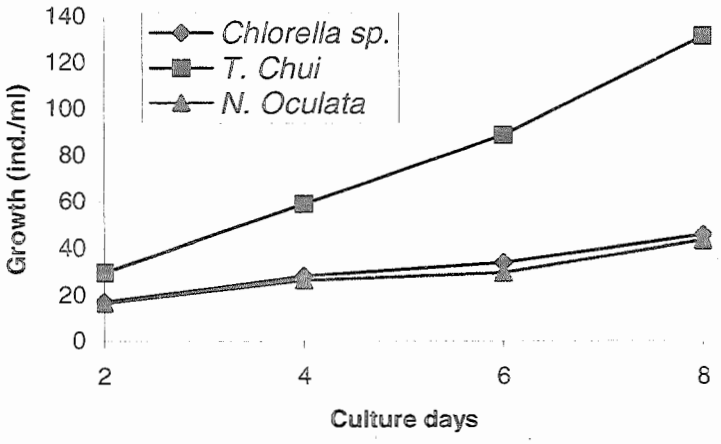

Fig. 1. Increment in growth of *B. plicatilis* fed on different microalge.

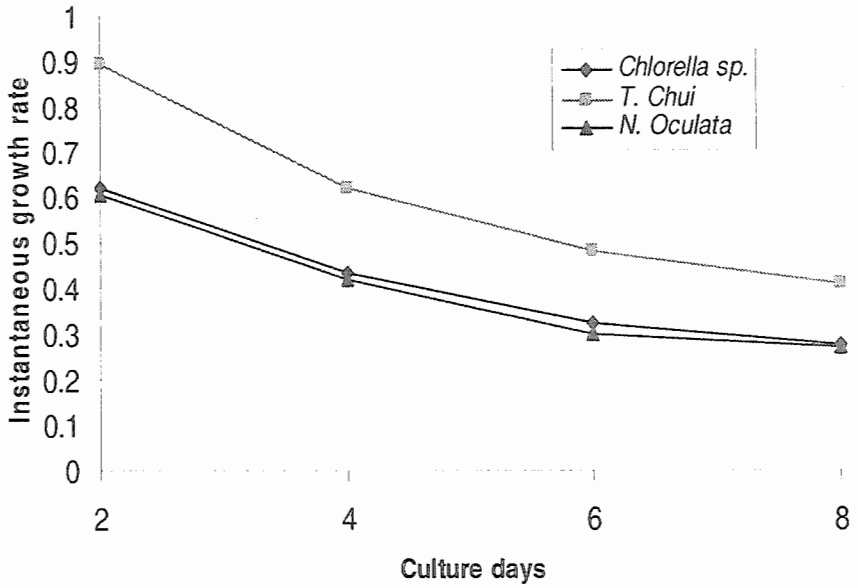

Fig. 2. Instantaneous growth rate in *B. plicatilis* fed on different microalgal diets.

The results of the present experiment is consistent with Braley (1994), who conducted experiments on stock culture of rotifer *B. plicatilis* in 600 ml flasks and reported a final density of 103 ind./ml at four days of culture, while feeding with *T. chui* at density of 4-5 x 10^6 cells /ml. The author also estimated about 38% of *T. chui* fed rotifers carrying eggs. Okauchi and Fukusho (1984) also reported *Tetraselmis* as a better marine rotifer food than *Chlorella* sp., due to not only its ease in mass culture, but also its capacity of transfer of favorable nutritional characteristics to marine rotifers making them excellent food for rearing many marine fish larvae.

Though bakers's yeast and some prepared feeds are used in feeding rotifer culture, microalgae have been found to produce the best growth. The common microalge that are being used widely are *Chlorella* sp., *Tetraselmis chui*, *N. oculata* and *I. galbana* (Hirayama *et al.*1979, Liao *et al.* 1991). However, different authors have reported the suitability of one species over the others at different culture conditions. Kongkeo (1991) observed rotifer density of 100 - 200 ind./ml after seven days of initial stocking (10-30 ind./ml) by feeding *N. oculata* (1-2 x 10^6 cells/ ml) and *T. chui* (2-4 x 10^4 cells/ ml) respectively under pure laboratory culture. Pi (1991) observed rotifer density of 200 ind./ml on the fifth day in mass culture by feeding *N. oculata* at the density of 2 x 10^7 cells/ ml. A rotifer density of 100-200 ind./ml, after four to five days of inoculation with initial density 30-50 ind./ml under mass culture condition, feeding *Chlorella* sp. at 8-10 x 10^6 cells/ ml has been reported by Park (1991). James *et al.* (1983) reported rotifer population density of 203 ind./ml, within seven days of mass culture from initial density of 80 ind./ml, by feeding *Chlorella* and Baker's yeast.

Villegas (1990) evaluated the effects of three selected algal species, *T. tetrathele, I. galbana and Chlorella* sp. on the population growth of *B. plicatilis* after 3, 5, and 7 days of culture. The rotifers fed on *T. tetrathele* showed superior growth with mean peak density of 92.5 ind./ml to those fed on *I. galbana* (48.2 ind./ml) and *Chlorella* sp. (47.2 ind./ml) in 5 days of culture. In consistent with this finding, it could be concluded from the results of the present study that *T. chui* might be an effective food species for stock culture of locally isolated rotifer *B.plicatilis* as well as for upscaling to mass culture of this zooplankton for larval rearing of crustaceans and finfish.

The available information of elsewhere and of the present study on effects of different microalgal diets on *B. plicatilis* culture indicate that the suitability of a particular microalgae species in rotifer culture largely depends on location of origin and nutritional quality. While marine *Chlorella* sp. has been considered better in the nutritional value than other species in Japan (Hirata *et al.* 1979), it has found nutritionally inferior to *N. oculata* in Taiwan aquaculture system (Liao *et al.* 1991). In spite of lower EPA content (4 – 8%), the nutritive value of *T. chui* for rotifers has been found higher than that of *N. oculata* (Liao *et al.* 1991). However, best growth in rotifer culture may be obtained by secondary enrichment of *T. chui* fed rotifer population with *Nannochloropsis* sp. Besides the choice of suitable microalgae species, factors like temperature, salinity and feed concentration must be taken into well consideration, as all these affect the growth of both L- and S-type strains of rotifer. It has already been reported (Liao *et al.* 1991) that while *Tetraselmis* sp. are less sensitive to environmental stress, *Nannochloropsis* sp. must be cultured in greenhouse where temperature is kept below 30°C.

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